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(54) SULPHATED POLYSACCHARIDES WITH ETHYLENIC DOUBLE BOND AT END OF CHAIN

(71) PHARMINDUSTRIE

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(57) Claim

1. Mixtures of sulphated polysaccharides having the general structure of the polysaccharides constituting the heparin and of which the acid groups are in the free form or in a salt form, wherein the polysaccharides constituting the said mixtures possess an ethylenic double bond at one of the extremities of their chain, said mixtures having, in the form of the sodium salt, the following characteristics:

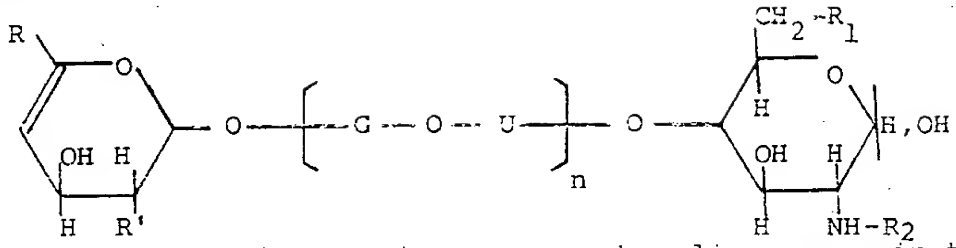
- percentage of sulphur : 9% to 13.5%
- percentage of nitrogen : 1.8% to 2.5%
- percentage of uronic acids: 20% to 30%
- weight mean molecular weight : 2000 to 10,000 daltons
- specific rotatory power in aqueous solution at 20°C:

$[\alpha]_D^{20}$: +25° to +55°

2. Mixtures according to claim 1, in which the polysaccharides constituting the said mixtures have the

.../2

formula:



(I)

wherein R is a hydrogen atom or a carboxylic group, in the free acid state or in the form of a salt, R' is an OH group or a sulphate group, in the free acid form or in the form of a salt, R_1 is an OH group or a sulphate group, in the free acid form or in the form of a salt, R_2 is a sulfonic group, in the free acid form or in the form of a salt, or an acetyl group, -O- is an oxygen bridge, the G linkages are the linkages of the glucosamine type appearing in the structure of heparin, the U linkages are the linkages of the uronic acid type (D-glucuronic acid, L-iduronic acid, sulphated L-iduronic acid) appearing in the structure of heparin, and n is a whole number from 3 to 20, the acid groups of said polysaccharides being in free form or in the form of a salt.

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Complete Specification for the invention entitled:

"NEW SULPHATED POLYSACCHARIDES, PROCESSES FOR THEIR PREPARATION AND THEIR USE AS MEDICAMENTS"

The following statement is a full description of this invention, including the best method of performing it known to us

NEW SULPHATED POLYSACCHARIDES, PROCESSES FOR THEIR PREPARATION AND THEIR USE AS MEDICAMENTS

The present invention relates to new mixtures of sulphated polysaccharides of weight mean molecular weight inferior to that of heparin, which can be used as anti coagulant and antithrombotic agents in the prevention and treatment of thromboses and as hypolipemiant agents.

Heparin is a mixture of sulphated mucopoly saccharides of animal origin, largely used for its anti coagulant and antithrombotic properties, notably in the prevention of the post-operative venous thromboses, and for its hypolipemiant properties. It is known that it acts in the coagulation process by activating a natural inhibitor of the coagulation contained in the blood, the antithrombin III. The activation of this protein has the effect of inhibiting the action of two protesses, the X-activated factor (factor Xa) on the one hand and the thrombin on the other.

The anticoagulant activity in vitro of the heparin is generally measured by the official methods of the pharmacopeia, especially of the American, English or French pharmacopeia, by referring to an international standard. But it is now possible to measure its specific activities in vitro, on the one hand with respect to the factor Xa and on the other hand with respect to thrombin (cf. for example A. TEIEN et al., Thrombosis Research, 11, 107, 1977).

The commercial heparins, which are mixtures of poly saccharides whose weight mean molecular weight is superior to 10,000 daltons and of which the dispersion of molecular weight goes from 4,000 to about 45,000 daltons, show the following activities in vitro:

Anticoagulant activity measured by the method of the French pharmacopeia, 8th edition, heparin monograph by referring to the 3rd international standard (or anticoagulant activity codex): 140 to 200 u.i./mg.

Anti-Xa activity measured by the method of TEIEN et al (already cited), by referring to the 3rd international standard: 160 to 180 u.i./mg.

A.P.T.T. activity (Activated Partial Thromboplastin Time) measured by the method of TEINEN et al (already cited), by referring to the 3rd international standard: 150 to 170 u.i./mg.

The heparin is administered obligatorily parenterally (in practice the subcutaneous way) and the action is relatively short, from which there are the two following important disadvantages: the need to carry out three administrations per day and the relatively high frequency of post-operative hemorrhagic accidents.

It is known (cf. JOHNSON et al, Thrombos. Haemostas. Stuttg. 1976, 35, 586-591; LANE et al, Thrombosis Research 16, 651-662, Pergamon Press Ltd. 1979; LASKER CHIU Annals N.Y. Acad. Sci., 222,7973, g71-977; LASKER Adv. Exp. Med. Biol. 52, 1975, 119-130) that, by fractionation of heparin, for example by filtration over Sephadox gel, fractions of mean molecular weight smaller than that of the heparin and having a dispersion of molecular weights smaller than that of heparin can be obtained.

The tests made in vitro and in vivo show that such fractions on the one hand are relatively more active on the X-activated factor than on the thrombin (that is that they show a $\frac{\text{anti-Xa activity}}{\text{anti-thrombinic activity}}$ ratio distinctly superior to 1), and on the other hand are more easily absorbed in the circulation from a subcutaneous injection than heparin itself, hence a higher and longer-lasting plasmatic activity than that of the heparin.

It is also known (cf. LASKER and CHIU already cited, LASKER already cited; U.S. Patent No. 3,766,167; PERLIN et al., Carbohydrate Research, 18, 1971, 185-194) to prepare, by enzymatic hydrolysis of heparin, depolymerisation products having a low mean molecular weight (practically 5,300 to 4,500 daltons, determined by ultracentrifuging) and an anti coagulating activity, determined by the method U.S.P. No. XVII, of about 70 u.i./mg. These products of depolymerisation can be fractionated, by filtration over Sephadex gel, into

fractions whose mean molecular weight, determined by ultracentrifuging, goes from 3200 to 5900 daltons and the anticoagulant activities from 45 to 95 u.i./mg (U.S.P. method). These depolymerisation products and the fractions which are obtained are active, taken orally as well as parenterally.

It is also known (cf. LASKER already cited) to prepare, by depolymerisation of heparin by means of ascorbic acid and hydrogen peroxide, products of low mean molecular weight. The depolymerisation by means of ascorbic acid and hydrogen peroxide leads, after fractionation in alcohol, to fractions having a mean molecular weight from 4000 to 7100 daltons and an anticoagulant activity of 12 to 100 u.i./mg (U.S.P. method).

Finally, it is known (cf. British Patent Application No. 2,002,406 A published on 21.02.1979) to prepare, by resulphatation of depolymerisation products of heparin devoid of anticoagulant activity, oligopolysaccharides having a mean molecular weight, determined by the Somogy method, of 2600 to 5500 daltons, a specific rotatory power, measured in aqueous solution at 20°C, of +40° to +50°, and an anticoagulant activity less than 50 u.i./mg (U.S.P. method), which would be as active taken orally as parenterally for the prevention of thromboses.

The mixtures of sulphated polysaccharides according to the invention are mixtures of sulphated polysaccharides having the general structure of the polysaccharides constitutive of heparin, that is showing the same elementary linkages as the polysaccharides constitutive of heparin, the said elementary linkages being linked in the same way as in the latter. However, contrary to the polysaccharides constitutive of heparin, the polysaccharides constitutive of the mixtures according to the invention comprise an ethylenic double band at one of the ends of the chain.

In the mixtures according to the invention, the acid groups of the sulphated polysaccharides may be in free form or in the form of a salt, in particular in the form of a sodium, calcium or magnesium salt.

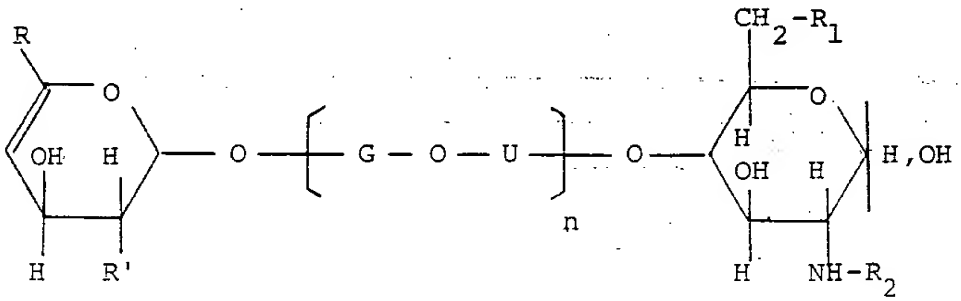
The mixtures according to the invention show, in the form of the sodium salt, the following characteristics:

- percentage of sulphur: 9% to 13.5%
- percentage of nitrogen: 1.8% to 2.5%
- percentage of uronic acids: 20% to 30%
- weight mean molecular weight: 2000 to 10,000 daltons
- specific rotatory power in aqueous solution at $20^{\circ}C$:

$[\alpha]_D^{20}$: $+25^{\circ}$ to $+55^{\circ}$

The sulphur and nitrogen contents and the rotatory power indicated above have been determined by the methods of the French pharmacopeia, 8th edition, heparin monograph. The contents of uronic acids has been determined by the method of N. BLUMENKRANTZ et al. (Analytical Biochemistry, 54, 484, 1973. The weight mean molecular weight has been determined by gel permeation chromatography on gel of polyacrylamide agarose by referring to the standard constituted by heparins of known mean molecular weight, according to the method of ANDERSON et al. (Thrombosis Research, 9, 575, 1976).

The polysaccharides constitutive of the mixtures according to the invention particularly correspond to the following formula:



(I)

wherein R is a hydrogen atom or a carboxylic group, in the free acid state or in the form of a salt, R' is an OH group or a sulphate group, in the free acid form or in the form of a salt, R_1 is an OH group or a sulphate group, in the free

acid form or in the form of a salt, R_2 is a sulfonic group, in the free acid form or in the form of a salt, or an acetyl group, -O- is an oxygen bridge, the G linkages are the linkages of the glucosamine type appearing in the structure of heparin, the U linkages are the linkages of the uronic acid type (D-glucuronic acid, L-iduronic acid, sulphated L-iduronic acid) appearing in the structure of heparin, and n is a whole number from 3 to 20.

In the above formula (I), the acid groups of the polysaccharides may be in free form or in the form of a salt, particularly a sodium, calcium or magnesium salt.

Although the present invention relates to all the mixtures as previously defined, it has more particularly as its object those of these mixtures which, in the form of the sodium salt, show the additional characteristics indicated for the catagories I, II, III in the Table A which follows:

TABLE A

	Category I	Category II	Category III
Weight mean molecular weight (in daltons)	8000 to 10,000	3000 to 8000	2000 to 7000
Anticoagulant activity in vitro Codex	130 to 160	80 to 140	10 to 80
Activity in vitro anti-Xa	130 to 180	120 to 250	80 to 250
Activity in vitro A.P.T.T.	100 to 150	80 to 120	10 to 80
Ratio $\frac{\text{Activity in vitro anti-Xa}}{\text{Activity in vitro A.P.T.T.}}$	1 to 1.5	1.4 to 3	2 to 10

The anticoagulant activity Codex indicated in the Table above has been determined by the method of the French pharmacopeia, 8th edition, heparin monograph. The anti-Xa and A.P.T.T. activities indicated in table A as well as those indicated in tables B and C further on have been determined by the method of TEIEN et al., (already cited) according to the following procedures:

a) Determination of the anti-Xa activity:

This activity is determined on an ox plasma, free of platelets, by means of the chromogenic substrate S 2222 [chromogenic peptide having the structure : (N-benzoyl)Ile-Glu-Gly-Arg-(p-nitro)anilide], refering to the 3rd international standard.

100 µl of citrated ox plasma, diluted from 2 to 5 with an aqueous buffer tris/EDTA pH 8.4, are added to 100 µl

of a solution of the product to be tested or of the standard in an aqueous buffer tris/EDTA pH 8.4, said 100 µl corresponding to 0.02 to 0.08 µg of product or standard. After 3 minutes of incubation at 37°C, 100 µl of an aqueous solution of Xa factor from ox, which correspond to 7n Kat of factor Xa, are added. After a 30 seconds incubation period, 200 µl of an aqueous solution of S 2222, which correspond to 0.2 µmole of S 2222, are added. After a three minutes incubation period, 300 µl of acetic acid are added and the optical density of the solution is measured at 405 nm in comparison with distilled water.

By plotting the optical density as a function of the concentration of product or standard, one obtains two straight lines, one relating to the product to be tested, the other relating to the standard. The activity of the product, expressed in international units per mg, is given by the formula:

$$173 \frac{\text{slope of the straight line relating to the product}}{\text{slope of the straight line relating to the standard}}$$

the number 173 corresponding to the value of the activity for the third international standard.

b) Determination of the A.P.T.T. activity

The product to be tested and the third international standard are dissolved in a 0.15 M aqueous solution of sodium chloride, then diluted with a citrated ox plasma, free of platelets, so as to obtain concentrations of product to be tested (or of standard) from 0. to 4 µg/ml.

100 µl of the reagent "automated APTT Precibio" (reagent based on phospholipids of rabbit brain and micronized silica) are added to 100 µl of the solution so obtained. After a five minutes incubation period at 37°C, 100 µl of a 0.025 M aqueous solution of calcium chloride are added. The clotting time is measured by means of a Bio-Mérieux fibrometer.

By plotting the logarithm of the clotting time as a function of the concentration of the product or of the standard, one obtains two straight lines, one relating to the

product to be tested, the other relating to the standard. The activity of the product, expressed in international units per mg, is given by the formula:

$$173 \times \frac{\text{slope of the straight line relating to the product}}{\text{slope of the straight line relating to the standard}}$$

the number 173 corresponding to the value of the activity for the third international standard.

All activities appearing in the Tables A, B and C are expressed in international units per mg (u.i./mg), referring to the 3rd international standard.

The mixtures according to the invention are prepared by the action of a mineral or organic base on a heparin ester resulting from the partial or total esterification of the carboxylic acid groups of the heparin. In this ester the acid groups of the heparin which are not esterified (that is, the acid sulphate groups and possibly a part of the carboxylic acid groups) may be in the free state or in the form of salts, in particular an alkali metal salt such as the sodium salt, an alkaline earth salt such as the calcium salt, a magnesium salt or quaternary ammonium salt with a long chain such as the benzethonium salt.

In the case where the heparin ester is soluble in water (for example where the not esterified acid groups are in the form of the sodium salt), the reaction between the ester and the base may be effected in water, at a temperature of 20°C to 80°C, the molar concentration of the base in the medium being preferably between 0.1 and 0.6. Bases which may be used are the bases soluble in water and in particular sodium hydroxide, potassium hydroxide, alkali metal carbonates, triethylamine, triethylenediamine, quinuclidine, 1,5-diazabicyclo [4.3.0] 5-nonene and 1,5-diaza-bicyclo [5.4.0.] 5-undecene. Once the reaction finishes, the product of depolymerisation formed is isolated, for example by precipitation by addition of sodium chloride then methanol.

The reaction between the ester and the base may also be effected, particularly when the not esterified acid groups of the ester are in the form of quaternary ammonium salt with a long chain, in an inert organic solvent for the said ester,

such as for example dichloromethane, dimethylformamide, formamide or tetrahydrofuran, at a temperature preferably of 20°C to 80°C. Bases which may be used are the bases soluble in the solvent used and in particular 1,5-diaza-bicyclo [4,3.0] 5-nonene, quinuclidine and 1,5-diaza-bicyclo [5.4.0] 5-undecene. Once the reaction has ended, the product of depolymerisation formed in which the carboxylic acid groups are still esterified is isolated in the form of an alkaline salt, and is hydrolysed by an aqueous solution of alkali metal hydroxide, especially sodium hydroxide, at least 1N, at low temperature (0°C to +5°C). The final product is separated, for example by precipitation by addition of sodium chloride then methanol.

Heparin esters which may be used as starting products for preparing the mixtures of polysaccharides according to the invention may be non-selective esters or selective esters. By non-selective esters are intended heparin esters wherein the carboxyl groups of the D-glucuronic acid, unsulphated L-iduronic acid and sulphated L-iduronic acid linkages are indiscriminately esterified. By selective esters are intended heparin esters wherein are esterified, partially or wholly, either only the carboxyl groups of the D-glucuronic acid linkages or only the carboxyl groups of the D-glucuronic acid and unsulphated L-iduronic acid linkages, or only the carboxyl groups of the unsulphated L-iduronic acid and sulphated L-iduronic acid linkages, or only the carboxyl groups of the sulphated L-iduronic acid linkages.

Heparin esters which may be used as starting products in the processes according to the invention are in particular the heparin esters described in the French Patent No. 2,150,724 and in the British Patent No. 1,501,095, as well as the methyl, ethyl, ethoxycarbonylmethyl, cyanomethyl, benzyl and substituted benzyl (especially 4-chloro-benzyl, 4-nitro-benzyl) esters of heparin. There are preferably employed as starting product the benzyl or substituted benzyl esters of heparin. The heparin esters used as starting

substances in the processes according to the invention may come from heparin of any origin (ox lung heparin, heparin from pigs mucous membrane, heparin from cattle intestines, etc...).

The non-selective methyl, ethyl, ethoxycarbonylmethyl, cyanomethyl, benzyl and substituted benzyl esters of heparin may be obtained, for example, by the action of a neutral quaternary ammonium or amino salt of heparin with a halogenated derivative of formula Hal - CH_2 - R(II), in which Hal represents a chlorine, bromine or iodine atom and R represents a hydrogen atom or a methyl, ethoxycarbonyl, cyano, phenyl or substituted phenyl group. This reaction is effected in solution or in suspension in an inert solvent such as dimethyl formamide, methylene chloride, dimethylsulphoxide, tetrahydrofuran or acetone, at a temperature between -20°C and +60°C.

The methyl, ethyl, ethoxycarbonylmethyl, cyanomethyl, benzyl and substituted benzyl esters of heparin. wherein are esterified, partially or wholly, either only the carboxyl groups of the D-glucuronic acid linkages or only the carboxyl groups of the D-glucuronic acid and unsulphated L-iduronic acid linkages are obtained by reacting a halogen derivative of the above formula (II) with an acid quaternary ammonium salt of heparin in which are salified, besides the sulfate groups, either only the carboxyl groups of the D-glucuronic acid linkages, or only the carboxyl groups of the D-glucuronic acid and unsulphated L-iduronic acid linkages, the other carboxyl groups being in the free acid form. This reaction is carried out in the same conditions as the reaction of the halogen derivative of the formula (II) with a neutral quaternary ammonium salt of heparin.

The acid quaternary ammonium salts of heparin, in which are salified, besides the sulfate groups, only the carboxyl groups of the D-glucuronic acid linkages, are prepared by reacting a quaternary ammonium salt with heparin, in an aqueous medium the pH of which is between 3 and 4.

The acid quaternary ammonium salts of heparin, in which are salified, besides the sulfate groups, only the carboxyl groups of the D-glucuronic acid and unsulphated L-iduronic acid linkages, are obtained by reacting a quaternary ammonium salt with heparin, in an aqueous medium the pH of which is low enough to form the quaternary ammonium salt of heparin wherein only the sulfate groups are salified (practically the pH is from 2 to 2.5), then selectively neutralising the carboxyl groups of the D-glucuronic acid and unsulphated L-iduronic acid linkages of the product so obtained by addition of a determined amount of quaternary ammonium hydroxide, in a dimethylformamide medium.

The amount of quaternary ammonium hydroxide to be added is deduced from the neutralisation curve in a dimethylformamide medium for a sample of the said product having a known weight.

The methyl, ethyl, ethoxycarbonylmethyl, cyanomethyl, benzyl and substituted benzyl esters of heparin wherein are esterified, partially or wholly, either only the carboxyl groups of the sulphated L-iduronic acid linkages or only the carboxyl groups of the unsulphated L-iduronic acid and sulphated L-iduronic acid linkages, are prepared by reacting an alcohol of the formula HO - CH_2 - R(III), in which R is a hydrogen atom or a methyl, ethoxycarbonyl, cyano, phenyl or substituted phenyl group, with heparin, in an aqueous medium, in presence of a water-soluble condensation agent of the carbodiimide type such as, for example, 1-ethyl-3-(3-dimethyl-eminopropyl) - carbodiimide, the pH of the medium being adjusted to a value in the range 3.5 - 4.5 in the first case and in the range 2-3 in the second case. As alcohol of formula (III) which can be used, may be particularly mentioned methanol and ethanol, in which case one obtains respectively a selective methyl ester of heparin and a selective ethyl ester of heparin.

The processes according to the invention using the action of a mineral or organic base on a heparin ester, which are processes by β-elimination, allow to be obtained a partial and controlled depolymerisation of the heparin

without altering its general structure.

The mixtures of sulphated polysaccharides according to the invention possess anticoagulant and antithrombotic activities and a hypolipemiant activity. For mixtures having a sufficiently low mean molecular weight (in practice less than or equal to 7000 daltons), the antithrombotic activity is greater than the anticoagulant activity. The mixtures according to the invention have little toxicity. For example the product of example 9 is not toxic at a dose 300 mg/kg when administered intravenously to rats and mice. When administered subcutaneously, its toxicity is equal to that of heparin.

The mixtures of sulphated polysaccharides according to the invention in which the acid groups of the polysaccharides are in the form of pharmaceutically acceptable salts, especially in the form of the sodium, calcium or magnesium salt, can be used, as anticoagulant and antithrombotic agents, for the prevention and treatment of thrombosis. They are also utilisable for the treatment of hyperlipemia. They can be advantageously substituted for heparin for such applications. In fact, when administered subcutaneously, they show a longer-lasting action than heparin, which enables the frequency of the injections to be reduced. Further, they provoke less secondary effects (Hemorrhagic effects than heparin.

They can be administered, in admixture with a pharmaceutically acceptable vehicle, intravenously, subcutanously, via the lungs (inhalation), rectum and, for the mixtures of sufficiently low mean molecular weight (mixtures of category III in particular), orally. The doses administered depend on the method of administration and on the desired effect (antithrombotic or hypolipemiant effect).

The following examples illustrate the invention without it being restricted theretc.

The neutral benzethonium salt of heparin or benzethonium heparinate used as the starting substance in the examples 1 to 3, 10, 12 to 15 comes from a pig's intestine

heparin having the following characteristics:

- weight mean molecular weight: 16,000 daltons
- specific rotatory power in aqueous solution at 20°C:

$$[\alpha]_D^{20} : +41^\circ$$

- anticoagulant activity Codex: 157 u.i./mg

The neutral benzethonium salt of heparin or benzethonium heparinate used as starting product in the Examples 4 to 9 and 11 comes from a heparin from cattle intestines having the following characteristics:

- mean molecular weight by weight: 11,400 daltons

$$[\alpha]_D^{20} : +37^\circ$$

- anticoagulant activity Codex: 128 u.i./mg

The neutral benzethonium salt of heparin or benzethonium heparinate used as the starting product in the example 16 comes from a pig's mucosa heparin having a weight mean molecular weight 16,000 daltons, a specific rotatory power in aqueous solution at 20°C of +44° and a Codex anticoagulant activity 180 u.i./mg.

The sodium salt of heparin used as starting product in examples 17 to 19 corresponds to the above pig's mucosa heparin.

EXAMPLE 1

30 g of (4-chloro)benzyl chloride are added to a solution of 30 g of benzethonium heparinate in 600 ml of dimethylformamide. After solution, the reactants are left in contact for 60 hours at the ambiant temperature (about 20°C), then 600 ml of a 10% solution of sodium acetate in methanol are added. The precipitate formed is separated by filtration, washed with methanol and dried in vacuo. 10.75 g of the 4-chloro-benzyl ester of heparin, in the form of the sodium salt, are thus obtained.

The ester obtained is contacted, with stirring, with 269 ml of a 0.4N aqueous solution of sodium hydroxide at 25°C. At the end of 2 hours, neutralisation is effected by

addition of a 0.4N aqueous solution of hydrochloric acid and precipitation is effected by addition of two volumes (that is, double the volume of the aqueous phase) of methanol. 8.46 g of depolymerised heparin in the form of the sodium salt are isolated by filtration.

EXAMPLE 2

30 g of benzyl chloride are added to a solution of 30 g of benzethonium heparinate in 600 ml of dimethylformamide. After solution, the reactants are left in contact for 60 hours at the ambiant temperature, and precipitation is effected by addition of 600 ml of a 10% solution of sodium acetate in methanol. The precipitate is isolated by filtration, washed with methanol and dried in vacuo. 11.4 g of the benzyl ester of heparin is thus obtained, in the form of the sodium salt.

3g of the above ester are left in contact for 2 hours, with stirring, with 75 ml of a 0.4N aqueous solution of sodium hydroxide at 20°C-25°C. Then the solution is neutralised by addition of a 0.4N aqueous solution of hydrochloric acid and precipitation is effected by adding 2 volumes of methanol. 2.23 g of depolymerised heparin are thus isolated, in the form of the sodium salt.

EXAMPLE 3

10 g of benzyl chloride are added to a solution of 10 g of benzethonium heparinate in 250 ml of dichloromethane.

After solution, the mixture is left for 24 hours at the ambiant temperature, then the solvent is evaporated in vacuo. The residue is dissolved in 150 ml of dimethylformamide and precipitation is effected by addition of 150 ml of a 10% solution of sodium acetate in methanol. 3.67 g of the benzyl ester of heparin are separated by filtration, in the form of the sodium salt.

2 g of the above ester are treated for 2 hours, with stirring, with 50 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C. After cooling, the solution is neutralised by addition of a 0.1N aqueous solution in hydrochloric acid, then precipitation is effected by addition of 2 volumes of methanol. 1.54 g of depolymerised heparin are

thus obtained, in the form of the sodium salt.

EXAMPLE 4

5 g of ethyl chloracetate are added to a solution of 5 g of benzethonium heparinate in 125 ml of dichloromethane and, after solution, the substances are left in contact for 3 days at the ambiant temperature. The solvent is evaporated under vacuum, the residue is taken up with 75 ml of dimethylformamide and precipitation is effected by addition of 75 ml of a 10% solution of sodium acetate in methanol. The precipitate, separated by filtration, is washed in methanol, then dried in vacuo. 1.72 g of the carbethoxymethyl ester of heparin are thus obtained, in the form of the sodium salt.

1.7 g of the above ester are treated with 43 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C, with stirring, for two hours. After cooling, the solution is neutralised by addition of a 0.1N aqueous solution of hydrochloric acid and precipitation is effected by addition of two volumes of methanol. 1.33 g of depolymerised heparin are isolated by filtration, in the form of the sodium salt.

EXAMPLE 5

10 g of (4-chloro)benzyl chloride are added to a solution of 10 g of benzethonium heparinate in 250 ml of dichloromethane, and dissolved by stirring. The solution is left for 24 hours at the ambiant temperature, then the solvent is evaporated in vacuo. The residue is taken up with 150 ml of dimethylformamide and precipitation is effected by addition of 150 ml of a 10% solution of sodium acetate in methanol. After filtration, washing the precipitate with methanol and drying in vacuo, 3.84 g of the (4-chloro)-benzyl ester of heparin are isolated in the form of the sodium salt.

2 g of the above ester are treated with 50 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C, with stirring, for two hours. After cooling and neutralisation with a 0.1N aqueous solution of hydrochloric acid, precipitation is effected by addition of two volumes of methanol. The precipitate is isolated by filtration, washed with methanol and dried in vacuo. 1.38 g of depolymerised

heparin are thus obtained, in the form of the sodium salt.

EXAMPLE 6

5 g of (4-nitro)benzyl chloride are added to a solution of 5 g of benzethonium heparinate in 125 ml of dichloromethane and dissolved by stirring. The solution is then left for 3 days at the ambiant temperature, then the solvent is evaporated in vacuo and the residue is dissolved in 75 ml of dimethylformamide. The ester formed is precipitated by addition of 75 ml of a 10% solution of sodium acetate in methanol. The precipitate is collected by filtration, washed in methanol and dried in vacuo. 1.89 g of the (4-nitro)-benzyl ester of heparin are thus obtained, in the form of the sodium salt.

1.85 g of the above ester are treated with 46 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C for two hours, with stirring. After cooling, neutralisation is effected by addition of a 0.1N aqueous solution of hydrochloric acid, and precipitation is effected by addition of two volumes of methanol. The precipitate is isolated by filtration, washed with methanol and dried in vacuo. 1.13 g of depolymerised heparin are thus obtained, in the form of the sodium salt.

EXAMPLE 7

30 g of benzyl chloride are added to a solution of 30 g of benzethonium heparinate in 500 ml of dichloromethane and dissolved by stirring. The solution is then left at ambiant temperature for 24 hours, then the solvent is evaporated in vacuo and the residue is taken up with 400 ml of ether. The insoluble material is separated by filtration. 30 g of the benzyl ester of heparin are thus obtained, in the form of the benzethonium salt. This ester is dissolved in 200 ml of dichloromethane containing 8 ml of 1,5-diaza-bicyclo [4.3.0]5-nonene. The solution is refluxed for 3h 30, then the solvent is evaporated in vacuo. The residue is dissolved in 450 ml of dimethylformamide and an equal volume of a 10% solution of sodium acetate in methanol is added. The precipitate is collected and washed in methanol. Then it is treated at 0°C for an hour by a 1N aqueous solution of

sodium hydroxide. After neutralisation, precipitation is effected by addition of two volumes of methanol. The precipitate is isolated by filtration, washed with methanol and dried in vacuo.

6.6 g of depolymerised heparin are obtained, in the form of the sodium salt.

EXAMPLE 8

10 g of (4-chloro)benzyl chloride are added to a solution of 10 g of benzethonium heparinate in 250 ml of dichloromethane and dissolved by stirring. The solution is left at ambient temperature for 24 hours, then the solvent is evaporated in vacuo. The residue is taken up with 200 ml of ether and the precipitate formed is isolated by filtration. 10 g of the (4-chloro)-benzyl ester of heparin are thus obtained, in the form of the benzethonium salt.

5 g of this product are dissolved in 100 ml of dichloromethane containing 1.5 ml of 1,5-diaza-bicyclo [4.3.0] 5-nonene and refluxed for 4 hours. The then solvent is evaporated in vacuo, the residue is taken up with 30 ml of dimethylformamide and 100 ml of a 10% solution of sodium acetate in methanol are added. The precipitate formed is isolated by filtration, washed with methanol, then treated by 24 ml of a 1N aqueous solution of sodium hydroxide for an hour at 0°C. The solution is neutralised by addition of a 1N aqueous solution of hydrochloric acid and precipitation is effected by addition of two volumes of methanol. The precipitate is separated by filtration. After washing with methanol and drying in vacuo, 1 g of depolymerised heparin is obtained, in the form of the sodium salt.

EXAMPLE 9

30 g of benzyl chloride are added to a solution of 30 g of benzethonium heparinate in 600 ml of dimethylformamide. After solution, the reactants are left in contact for 60 hours at the ambiant temperature, then the ester formed is precipitated by addition of 1200 ml of a 10% solution of sodium acetate in methanol. The precipitate is isolated by filtration, washed with methanol and dried in

vacuo. 11.4 g of the benzyl ester of heparin are thus obtained, in the form of the sodium salt.

10 g of the above ester are treated with 250 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C, for two hours with stirring. After cooling, the solution is neutralised by a 0.1N aqueous solution of hydrochloric acid and precipitation is effected by addition of two volumes of methanol. The precipitate is filtered, washed with methanol and dried in vacuo. 6.65 g of depolymerised heparin are thus obtained, in the form of the sodium salt.

EXAMPLE 10

120 g of (4-chloro)benzyl chloride are added to a solution of 120 g of benzethonium heparinate in 2.4 l of dimethylformamide and dissolved by stirring. The solution is then left at ambiant temperature for 60 hours, then 2.4 l of a 10% solution of sodium acetate in methanol are added. The precipitate formed is separated by filtration, washed with methanol and dried in vacuo. 46 g of the (4-chloro)-benzyl ester of heparin are thus obtained, in the form of the sodium salt.

20 g of the above ester are treated with 500 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C, for two hours, with stirring. After cooling and neutralisation, precipitation is effected by addition of two volumes of methanol. 11.7 g of depolymerised heparin are isolated by filtration in the form of the sodium salt.

EXAMPLE 11

30 g of methyl iodide are added to a solution of 30 g of benzethonium heparinate in 750 ml of dichloromethane and dissolved by stirring. The solution is left for 48 hours at the ambiant temperature, then the solvent is evaporated in vacuo. The residue is taken up with 450 ml of dimethylformamide and precipitation is effected by addition of 450 ml of a 10% solution of sodium acetate in methanol. After filtration, the precipitate is washed with methanol and dried in vacuo. 10.5 g of the methyl ester of heparin are thus isolated in the form of the sodium salt.

2 g of the above ester are treated with 50 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C, with stirring for two hours. After cooling, the pH of the solution is brought to about 4.5 by stirring with a carboxylic ion exchange resin in the H^+ form. The resin is then separated by filtration and washed with water. The collected aqueous phases are neutralised by addition of a dilute aqueous solution of sodium hydroxide, and are then lyophilised. 2 g of depolymerised heparin are thus obtained, in the form of the sodium salt.

EXAMPLE 12

3 g of the (4-chloro)-benzyl ester of heparin obtained in Example 10 are treated with 120 ml of a 10% aqueous solution of disodium carbonate at 60°C, for two hours, with stirring. After cooling, the solution is neutralised by a 0.4N aqueous solution of hydrochloric acid and precipitation is effected by addition of two volumes of methanol. 1.57 g of depolymerised heparin are isolated by filtration, in the form of the sodium salt.

EXAMPLE 13

30 g of ethyl chloracetate are added to a solution of 30 g of benzethonium heparinate in 600 ml of dimethylformamide. After solution, the substances are left in contact for 60 hours at the ambiant temperature, then 600 ml of a 10% solution of sodium acetate in methanol is added. The precipitate formed is separated by filtration, washed with methanol and dried in vacuo. 10.78 g of the carbethoxymethyl ester of heparin are thus obtained, in the form of the sodium salt.

3 g of the above ester are put in contact with 100 ml of a 3% aqueous solution of triethylamine at a temperature of 60°C. At the end of 5 hours, the solution is neutralised by addition of an aqueous solution of hydrochloric acid, then precipitation is effected by addition of 2 volumes of methanol. 2.5 g of depolymerised heparin are isolated by filtration, in the form of the sodium salt.

EXAMPLE 14

5 g of chloracetonitrile are added to a solution of

5 g of benzethonium heparinate in 125 ml of dichloromethane and dissolved by stirring. The solution is left for 48 hours at the ambiant temperature, then the solvent is evaporated in vacuo. The residue is dissolved in 75 ml of dimethylformamide and precipitation is effected by addition of 75 ml of a 10% solution of sodium acetate in methanol. The precipitate is separated by filtration, washed with methanol and dried in vacuo. 1.63 g of the cyanomethyl ester of heparin are thus obtained, in the form of the sodium salt.

The ester obtained is treated with 40 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C, with stirring, for two hours. After cooling, the solution is neutralised by addition of a 0.1N aqueous solution of hydrochloric acid, then precipitation is effected by addition of two volumes of methanol. 1.33 g of depolymerised heparin are isolated by filtration, in the form of the sodium salt.

EXAMPLE 15

3 g of the (4-chloro)-benzyl ester of heparin obtained in Example 10 are dissolved, with stirring, in 120 ml of a 10% aqueous solution of disodium carbonate. After two hours stirring at a temperature from 20°C to 25°C, the pH of the solution is brought to 6 by addition of a N aqueous solution of hydrochloric acid, then a volume of methanol equal to twice the volume of the aqueous solution is added. The precipitate formed is isolated by filtration and 2.1 g of the (4-chloro)-benzyl ester of heparin are thus obtained.

2 g of the above ester are treated, with stirring, for two hours with 50 ml of a 0.1N aqueous solution of sodium hydroxide at 60°C. After cooling, the solution is neutralised by addition of a 0.1N aqueous solution of hydrochloric acid, then precipitation is effected by addition of two volumes of methanol. 1.4 g of depolymerised heparin are thus obtained, in the form of the sodium salt.

In the preceding Examples 1 to 10 and 12 to 15, before precipitating the product formed by addition of the two volumes of methanol, the concentration of NaCl in the aqueous phase was adjusted to 10% by addition of sodium

chloride.

EXAMPLE 16

10 g of (4-chloro)benzyl chloride are dissolved with stirring in a solution of 10 g of benzethonium heparinate in 250 ml of dichloromethane. The solution is left for 24 hours at the ambiant temperature, then a 10% solution of sodium acetate in methanol is added. The precipitate formed is filtered, washed with methanol. 3.72g of (4-chloro)benzyl ester of heparin are thus obtained, in the form of the sodium salt.

A solution of 0.500g of the above ester in 10ml of formamide is treated at 60°C, for 5 hours, with 0.5ml of 1,5-diaza-bicyclo[4,3,0]5-nonene. After cooling, 70 ml of acetone are added. 0.364g of a precipitate are collected by filtration. This precipitate is treated at 0°C, for two hours, with 6 ml of a N aqueous solution of sodium hydroxide. The aqueous phase is neutralised by addition of an N aqueous solution of hydrochloric acid and the concentration of NaCl in the medium is adjusted to 10% by addition of sodium chloride. Precipitation is effected by adding two volumes of methanol. 0.263g of depolymerised heparin are thus obtained, in the form of the sodiuim salt.

EXAMPLE 17

2.5 ml of acetic acid then, slowly and with stirring, 150 ml of a 10% aqueous solution of benzethonium chloride are added to a solution of 10 g of heparin (sodium salt) in 40 ml of water. The precipitate formed is collected by centrifugation, washed with water and dried. 19.67 g of benzethonium acid heparinate are obtained.

11 g of the above product are dissolved in 110 ml of dimethylformamide and 11 g of (4-chloro)-benzyl chloride are added. The reactants are left in contact for 48 hours at the ambiant temperature, then 220 ml of a 10% solution of sodium acetate in methanol are added. The precipitate formed is isolated by filtration, washed with methanol and dried under vacuum. 4.70 g of (4-chloro)-benzyl ester of heparin are thus obtained in the form of the sodium salt.

4 g of the above ester are dissolved in 20ml of water and 40ml of a 20% aqueous solution of benzethonium chloride are slowly added with stirring. The precipitate formed is collected by centrifugation, washed with water and dried under vacuum. The (4-chloro)-benzyl ester of heparin is thus obtained, in the form of the benzethonium salt.

1g of the above ester (benzethonium salt) is dissolved in 20 ml of dimethylformamide and is treated with 1 ml of 1,5-diaza-bicyclo[4,3,0]5-nonene at 60°C for five hours. After cooling, 50ml of a 10% solution of sodium acetate in methanol are added. The precipitate formed (0.346g) is collected and treated at 0°C, for two hours, with 5.8ml of a N aqueous solution of sodium hydroxide. The aqueous phase is neutralised by addition of a N aqueous solution of hydrochloric acid and the concentration of NaCl in the medium is adjusted to 10% by addition of sodium chloride. Precipitation is effected by adding two volumes of methanol. The precipitate is filtered, washed with methanol. 0.253g of depolymerised heparin are thus obtained in the form of the sodium salt.

EXAMPLE 18

2.5 ml of formic acid then, slowly and with stirring, 150 ml of a 10% aqueous solution of benzethonium chloride are added to a solution of 10 g of heparin (sodium salt) in 40 ml of water. The precipitate is collected by centrifugation, washed with water and dried under vaccum. 20.5 g of benzethonium acid heparinate are thus obtained.

2.95 g of the above product are dissolved in 60 ml of dimethylformamide, then 5.9 ml of a 0.1 N solution of tetrabutylammonium hydroxide in a n-propanol/methanol mixture are added. After the addition of 2.95g of (4-chloro)-benzyl chloride, the solution is left for five days at the ambiant temperature. 74 ml of a 10% solution of sodium acetate in methanol are added. 1.32g of (4-chloro)-benzyl ester of heparin are isolated by filtration in the form of the sodium salt.

The above ester is dissolved in 6.4ml of water and 12.8 ml of a 20% aqueous solution of benzethonium chloride

are slowly added with stirring. The precipitate formed is collected by centrifugation, washed with water and dried under vacuum. The (4-chloro)-benzyl ester of heparin is thus obtained, in the form of the benzethonium salt.

1g of the above ester (benzethonium salt) is dissolved in 20ml of dichloromethane and 1ml of 1,5-diaza-bicyclo [4,3,0] 5-nonene is added. The solution is heated with reflux for 5 hours, then the solvent is evaporated under vacuum, the residue is taken up with 15ml of dimethylformamide and 20 ml of a 10% solution of sodium acetate in methanol are added. The precipitate formed is separated by filtration and washed with methanol. 0.405g are thus obtained of a product which is treated at 0°C, for two hours, with 6 ml of a N aqueous solution of sodium hydroxide. The aqueous phase is neutralised by addition of a N aqueous solution of hydrochloric acid and the concentration of NaCl in the medium is adjusted to 10% by addition of sodium chloride. The precipitation is effected by adding two volumes of methanol. The precipitate is separated by filtration and washed with methanol. 0.355g of depolymerised heparin are thus obtained, in the form of the sodium salt.

EXAMPLE 19

0.600 g of heparin (sodium salt) are dissolved in 7ml of water and the pH of the solution is adjusted to 3.5 by addition of a N aqueous solution of hydrochloric acid. 0.300g of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide are added and, after dissolution, the solution is left for one hour at the ambiant temperature. 2.5ml of an aqueous solution of sodium chloride containing 280g per litre, then 15ml of methanol are added. The precipitate formed is isolated by filtration, washed with methanol and dried under vacuum. 0.527g of methyl ester of heparin, the esterification percentage of the carboxyl groups of which is 50%, are thus obtained in the form of the sodium salt.

0.300g of the above ester are dissolved in 7.5ml of a 0.1 N aqueous solution of sodium hydroxide and the solution is heated at 60°C for 2 hours. After cooling, the solution

is neutralised by addition of a 0.1 N aqueous solution of hydrocloric acid, the concentration of NaCl in the medium is adjusted to 10% by adding sodium chloride and the precipitation is effected by adding two volumes of methanol. 0.200g of depolymerised heparin are thus obtained in the form of the sodium salt.

The following Tables B and C give the characteristics of the products (depolymerised heparins in the form of the sodium salt) prepared in the Examples 1 to 19. The percentages of sulphur, nitrogen and uronic acids, the specific rotatory powers in aqueous solution at 20°C, the weight mean molecular weights and the activities indicated in these Tables have been determined by the methods previously described. In the column dispersion of molecular weights are given the approximative extreme values of the molecular weights of the polysaccharides constituting the mixtures, such as determined by gel permeation chromatography on gel of polyacrylamide-agarose. In the viscosity column, the viscosities at 25°C of the 10% aqueous solutions of the products are shown. In the UV absorption column are shown the absorptions by a 1cm thickness of the 1% solutions of the products in 0.01 N HCl, said absorptions being measured at the wave-length of the absorption maximum appearing in the range 220 - 232 nm.

TABLE C

Example	Mean Molecular Weight (in daltons)	Absorption UV	Anticoagulant Activity		
			anti-X a in vitro	A.P.T.T. in vitro	Codex in vitro
16	4200	6	140	63	60
17	5500	7.96	165	80	70
18	4400	6.3	110	60	40
19	5000	10.9	150	45	50

TABLE B

Example	% uronic	% S	% N	$[\alpha]_D^{20}$	Mean Molecular Weight (in daltons)	Dispersion of Molecular Weights
1	23.0	11.5	2.1	+44°	7300	1600 - 12,000
2	26.0	11.6	2.0	+44°	6500	1800 - 13,000
3	25.4	12.0	2.2	+44°	6400	3000 - 11,000
4	23.9	10.9	2.1	+35°	5500	2200 - 13,000
5	22.8	12.2	2.2	+39°	5000	2000 - 13,000
6	24.5	10.8	2.3	+33°	4200	1000 - 10,000
7	25.2	11.3	2.0	+33°	4100	1700 - 10,000
8	23.7	11.1	2.2	+35°	4500	1000 - 10,000
9	26.0	11.8	2.2	+38°	4000	2000 - 10,000
10	25.2	10.9	2.1	+41°	3800	1000 - 9,000
11	23.8	11.0	2.3	+27°	2800	1000 - 8,000
12	23.9	11.5	2.0	+40°	4000	2000 - 9,000
13	24.3	11.8	2.2	+45°	9000	3000 - 20,000
14	25.6	12.0	2.1	+44°	8500	3000 - 20,000
15	25.3	11.8	2.3	+45°	4000	2000 - 20,000

TABLE B (continued)

Example	Viscosity (in centipoises)	Absorption UV	Anticoagulant Activity Codex in vitro (u.i./mg)	Anti-Xa activity in vitro (u.i./mg)	A.P.T.T. activity in vitro (u.i./mg)
1	2.37	7.5	127	180	105
2	2.25	7.8	116	148	100
3	2.08	7.9	112	163	89
4	1.93	8.2	83	130	52
5	1.91	9.7	75	118	43
6	1.74	12.5	60	140	33
7	1.64	15.0	50	130	30
8	1.61	16.4	54	110	33
9	1.62	14.0	80	159	45
10	1.57	15.4	62	159	40
11	1.50	22.8	40	90	20
12	1.60	14.4	75	140	35
13	2.60	5.9	135	140	130
14	2.50	6.1	138	130	130
15	1.65	10.64	110	170	86

EXAMPLE 20

The product of Example 1 on the one hand and a commercial heparin on the other hand have been administered, at different times, by subcutaneous way, to five healthy volunteers, at a dose of 5000 u.i. Codex. On the blood samples taken 1 hour, 3 hours, 5 hours and 7 hours after the administration, there was measured the plasmatic anticoagulant activity by means of the anti -Xa and A.P.T.T. tests previously defined. The results obtained have been expressed in international units per ml of plasma, by referring to a standard curve traced from tests effected on a control plasma to which has been added known quantities of reference heparin (3rd international standard).

The average results obtained are collected in the following Table D:

TABLE D

Time elapsing between the administration and the sampling	Anti-Xa activity		A.P.T.T. activity	
	Product of Example 1	Commercial Heparin	Product of Example 1	Commercial Heparin
1 hour	0.20	0.04	0.04	0.05
3 hours	0.27	0.08	0.05	0.04
5 hours	0.28	0.05	0.03	0.02
7 hours	0.22	0.05	0.02	0.01

It is seen that the product of Example 1 exerts an anti-Xa effect much more intense than that of the commercial heparin.

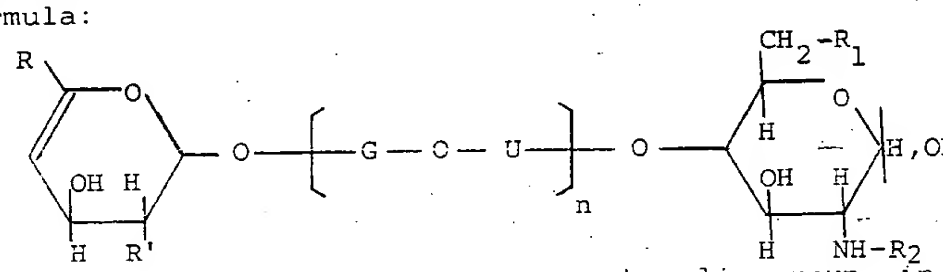
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. Mixtures of sulphated polysaccharides having the general structure of the polysaccharides constituting the heparin and of which the acid groups are in the free form or in a salt form, wherein the polysaccharides constituting the said mixtures possess an ethylenic double bond at one of the extremities of their chain, said mixtures having, in the form of the sodium salt, the following characteristics:

- percentage of sulphur : 9% to 13.5%
- percentage of nitrogen : 1.8% to 2.5%
- percentage of uronic acids: 20% to 30%
- weight mean molecular weight : 2000 to 10,000 daltons
- specific rotatory power in aqueous solution at 20°C:

$[\alpha]_D^{20}$: +25° to +55°

2. Mixtures according to claim 1, in which the polysaccharides constituting the said mixtures have the formula:



(I).

wherein R is a hydrogen atom or a carboxylic group, in the free acid state or in the form of a salt, R' is an OH group or a sulphate group, in the free acid form or in the form of a salt, R_1 is an OH group or a sulphate group, in the free acid form or in the form of a salt, R_2 is a sulfonic group, in the free acid form or in the form of a salt, or an acetyl group, -O- is an oxygen bridge, the G linkages are the linkages of the glucosamine type appearing in the structure of heparin, the U linkages are the linkages of the uronic acid type (D-glucuronic acid, L-iduronic acid, sulphated

L-iduronic acid) appearing in the structure of heparin, and n is a whole number from 3 to 20, the acid groups of said polysaccharides being in free form or in the form of a salt.

3. Mixtures according to Claims 1 or 2, which, in the form of the sodium salt, show the following characteristics:

- weight mean molecular weight : 8000 to 10,000 daltons
- anticoagulant activity in vitro Codex : 130 to 160 u.i./mg
- activity in vitro anti-Xa : 130 to 180 u.i./mg
- activity in vitro A.P.T.T. : 100 to 150 u.i./mg
- ratio $\frac{\text{activity in vitro anti-Xa}}{\text{activity in vitro A.P.T.T.}}$: 1 to 1.5

4. Mixtures according to Claim 1 or 2, which show, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight : 3000 to 8000 daltons
- anticoagulant activity in vitro Codex : 80 to 140 u.i./mg
- activity in vitro anti-Xa : 120 to 250 u.i./mg
- activity in vitro A.P.T.T. : 80 to 120 u.i./mg
- ratio $\frac{\text{activity in vitro anti-Xa}}{\text{activity in vitro A.P.T.T.}}$: 1.4 to 3

5. Mixtures according to Claims 1 or 2, which show, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight: 2000 to 7000 daltons
- anticoagulant activity in vitro Codex: 10 to 80 u.i./mg
- activity in vitro anti-Xa: 80 to 250 u.i./mg
- activity in vitro A.P.T.T.: 10 to 80 u.i./mg
- ratio $\frac{\text{activity in vitro anti-Xa}}{\text{activity in vitro A.P.T.T.}}$: 2 to 10.

6. Mixture according to Claim 5, which shows, in the

form of the sodium salt, the following characteristics:

- weight mean molecular weight: 4200 daltons
- rotatory power $[\alpha]_D^{20} = + 33^o$
- anticoagulant activity in vitro Codex: 60 u.i./mg
- activity in vitro anti-Xa: 140 u.i./mg
- activity in vitro A.P.T.T.: 33 u.i./mg

7. Mixture according to Claim 5 which shows, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight: 4100 daltons
- rotatory power $[\alpha]_D^{20} = + 33^o$
- anticoagulant activity in vitro Codex: 50 u.i./mg
- activity in vitro anti-Xa: 130 u.i./mg
- activity in vitro A.P.T.T.: 30 u.i./mg

8. Mixture according to Claim 5, which shows, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight: 4500 daltons
- rotatory power $[\alpha]_D^{20} = + 35^o$
- anticoagulant activity in vitro Codex: 54 u.i./mg
- activity in vitro anti-Xa: 110 u.i./mg
- activity in vitro A.P.T.T.: 33 u.i./mg

9. Mixture according to Claim 5, which shows, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight: 4000 daltons
- rotatory power $[\alpha]_D^{20} = + 38^o$
- anticoagulant activity in vitro Codex: 80 u.i./mg
- activity in vitro anti-Xa: 159 u.i./mg
- activity in vitro A.P.T.T.: 45 u.i./mg

10. Mixture according to Claim 5, which shows, in the form of the sodium salt, the following charcteristics:

- weight mean molecular weight: 3800 daltons

- rotatory power $[\alpha]_D^{20} = +41^{\circ}$
- anticoagulant activity in vitro Codex: 62 u.i./mg
- activity in vitro anti-Xa: 159 u.i./mg
- activity in vitro A.P.T.T.: 40 u.i./mg

11. Mixture according to Claim 5, which shows, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight: 2800 daltons
- rotatory power $[\alpha]_D^{20} = +27^{\circ}$
- anticoagulant activity in vitro Codex: 40 u.i./mg
- activity in vitro anti-Xa: 90 u.i./mg
- activity in vitro A.P.T.T.: 20 u.i./mg

12. Mixture according to Claim 5, which shows, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight: 4000 daltons
- rotatory power $[\alpha]_D^{20} = +40^{\circ}$
- anticoagulant activity in vitro Codex: 75 u.i./mg
- activity in vitro anti-Xa: 140 u.i./mg
- activity in vitro A.P.T.T.: 35 u.i./mg

13. Mixture according to Claim 4, which shows, in the form of the sodium salt, the following characteristics:

- weight mean molecular weight: 4000 daltons
- rotatory power $[\alpha]_D^{20} = +45^{\circ}$
- anticoagulant activity in vitro Codex: 110 u.i./mg
- activity in vitro anti-Xa: 170 u.i./mg
- activity in vitro A.P.T.T.: 86 u.i./mg

14. A process for the preparation of the mixtures according to Claim 1, in which a water-soluble heparin ester resulting from the partial or total esterification of the carboxylic acid groups of heparin is reacted, in aqueous medium, at a temperature from 20°C to 80°C, with a water

soluble mineral or organic base, and the product of depolymerisation thus formed is isolated.

15. A process according to Claim 14, in which the molar concentration of the base in the medium is 0.1 to 0.6.

16. A process for the preparation of the mixtures according to Claim 1, in which a heparin ester resulting from the partial or total esterification of the carboxylic acid groups of heparin is reacted with a base, in an inert organic solvent of the said ester, the product of depolymerisation thus formed is isolated as the alkali metal salt and hydrolysis is effected by an at least 0.1N aqueous solution of sodium hydroxide at low temperature.

17. A process according to Claim 16, in which the reaction of the heparin ester with the base is effected at a temperature from 20°C to 80°C.

18. A medicament, utilisable in particular for the prevention and treatment of thromboses and for the treatment of hyperlipemia, which contains as active ingredient a mixture of sulphated polysaccharides as defined in any one of claims 1 to 13, the acid groups of the said polysaccharides being in the form of pharmaceutically acceptable salts, and pharmaceutically acceptable diluent or carrier.

19. A medicament according to Claim 18, in which the acid groups of the sulphated polysaccharides are in the form of sodium salts, calcium salts or magnesium salts.

DATED this 11th day of May, 1981

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